Cell, Vol. 83, 13–15, October 6, 1995, Copyright © 1995 by Cell Press

Alterations in DNA Methylation May Play a Variety of Roles in Carcinogenesis

Minireview

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There is considerable interest in the role that DNA methylation (5-methylcytosine [5MeC] content of DNA) plays in both normal development (Razin and Kafri, 1994) and carcinogenesis (Laird et al., 1995). However, there are what at first glance may seem to be conflicting reports concerning the role of DNA methylation in carcinogenesis. We have emphasized the hypothesis that hypomethylation of DNA facilitates aberrant gene expression in tumorigenesis (Counts and Goodman, 1994). Others have supported the contention that hypermethylation of DNA leads to the causative alteration in tumorigenesis that involves inactivating tumor suppressor genes and marking chromosome regions for deletion (Herman et al., 1994). Still others have downplayed the importance of alterations in gene expression and favor mutation playing the key role (Laird et al., 1995). We believe that there is actually more harmony than discord here and that focusing attention singly on one mechanism may impede an overall understanding of carcinogenesis. In this minireview we juxtapose the view that carcinogenesis is a multistep/multistage process that occurs in a whole animal (Pitot and Dragan, 1991) with the notion that carcinogenesis is more than mutagenesis and indicate why one should expect DNA methylation to play multiple roles in the transformation of a normal cell into a frank malignancy. We feel that apparently disparate views can thus be reconciled in a fashion that provides insight regarding mechanisms underlying carcinogenesis.

Changes in DNA Methylation and Cancer

Both general hypomethylation and areas of regional hypermethylation coexist in the genome of a wide variety of human and animal cancers (Gama-Sosa et al., 1983; Herman et al., 1994; Counts and Goodman, 1994). This has made attempts to define a precise role for DNA methylation in carcinogenesis very difficult. Indeed, different effects may be evident depending upon the model system or target tissue. It has been suggested that changes in methylation may not play a causal role in carcinogenesis and could be a consequence of the transformed state of the tumor cell and that C to T transitions brought about by increased expression of the DNA methyltransferase (MTase) play the key role (e.g., Laird et al., 1995). While DNA MTase-induced mutations could play an important role, additional factors appear to be involved in light of the multiple steps and stages in the cancer process. Cancers originate from a single cell that is changed dramatically by a series of alterations to the genome, e.g., mutation and changes in methylation that alter gene expression. Clearly, mutagenesis plays a role in carcinogenesis. However, with the exception of tumor suppressor genes, a mutated gene must be expressed to have an effect.

Interestingly, two recent publications indicate that reduction of DNA MTase activity, which would be expected to result in marked hypomethylation, can inhibit tumorigenesis (MacLeod and Szyf, 1995; Laird et al., 1995). Since DNA methylation plays a pivotal role in development and differentiation (Li et al., 1993; Razin and Kafri, 1994), we believe it is reasonable to propose that hypomethylation at an intermediate level plays a critical role in carcinogenesis while excessive hypomethylation may not be compatible with the life of the affected cells (e.g., owing to massive deregulation of gene expression). By this we mean that initiated cells may die under the severe conditions of hypomethylation and thus would not be available to form tumors. Thus, the fact that inhibition of methylation may decrease tumor formation does not prove that excess DNA MTase activity is the sole mechanism underlying carcinogenesis.

Possible Roles for Alterations in DNA Methylation in Carcinogenesis

The well-established role for mutagens and mutagenesis in carcinogenesis must be reconciled with the fact that not all carcinogens are mutagens and the view that nonmutagenic events are also involved in transformation. This is illustrated by a recent study examining a mismatch repair deficiency that has been identified in phenotypically normal human cells. The people who donated these cells had numerous mutations in a variety of tissues, but, contrary to what would be expected if multiple mutations were solely responsible for carcinogenesis, they exhibited very few tumors (Parsons et al., 1995). There are mechanistically and theoretically plausible nongenotoxic mechanisms that support roles for both hypomethylation and hypermethylation of DNA (i.e., epigenetic changes) in carcinogenesis; these involve mainly alterations in normal gene expression (including tumor suppressor genes). Altered DNA methylation not only effects expression but also may facilitate mutation, as 5MeC can deaminate spontaneously to T. This means elevated expression of the DNA MTase may lead to increased 5MeC, and this can increase the probability of C to T transitions (Laird et al., 1995; Yang et al., 1995).

Hypomethylation of DNA is associated with increased gene expression (Razin and Kafri, 1994; Ferguson et al., 1995). This can play a role in carcinogenesis. For example, increased expression of mutated Ha-*ras* appears to be involved in transformation (Finney and Bishop, 1993). Additionally, a decreased capacity or fidelity of maintaining the normal methylation status of DNA may underlie the heightened sensitivity of some mouse strains to liver tumorigenesis, in which increased expression of oncogenes appears to be involved by facilitating tumor promotion (Ray et al., 1994; Counts and Goodman, 1994, 1995). The principal characteristic of the promotion stage of carcinogenesis that distinguishes it from the stages of initiation and progression is its operational reversibility. That is, clones of initiated cells regress when the promoting agent is withdrawn. The promotion stage ends when a lesion attains the

capacity for growth in the absence of a promotion stimulus (i.e., when it is no longer reversible) and can progress to a frank malignancy (PItot and Dragan, 1991). The presence of a mechanism for de novo methylation in the whole animal (Razin and Kafri, 1994) provides the potential to reverse hypomethylation; thus, its proposed role in tumor promotion that we and others support is consistent with the biological observation that reversibility is a hallmark of this stage of carcinogenesis and compatible with the whole animal situation. In this regard, hypomethylation may be one of the crucial factors that facilitates clonal expansion of the progenitor cells that lead to tumors. We have shown hypomethylation of a particular site in the *raf* gene during early stages of phenobarbital-induced mouse liver tumorigenesis that is maintained in its unmethylated state in the phenobarbital-induced tumors; thus, it appears cells from the population that initially exhibited hypomethylation have advanced to yield the tumors that formed (Ray et al., 1994).

Hypermethylation of specific regions of DNA has also been identified in cancer cells. For example, the VHL tumor suppressor gene is hypermethylated and inactivated in a fraction of renal cell lines and tumors that did not have mutations in the coding regions sequenced (Herman et al., 1994). Other investigators have demonstrated associations in tumors between regional chromosomal hypermethylation and areas believed to contain tumor suppressor genes at a variety of target sites (references can be found in Herman et al., 1994). This regional hypermethylation in portions of the genome normally unmethylated may inactivate tumor suppressor genes. The functional significance of this would be the same as an inactivating mutation or as the loss of an allele. In support of this, regional hypermethylation of the retinoblastoma gene appears to inhibit transcription of this tumor suppressor gene (Greger et al., 1994). It will be important to determine not only which tumor suppressor genes lie in specific regions and their normal functions, but also to demonstrate a causative role, perhaps by reversing their inactivation.

Another potential mechanism underlying a role of altered DNA methylation and carcinogenesis is the intrinsic hypermutability of 5MeC as compared with C via deamination; this leads to C to T transitions (references can be found in Yang et al., 1995). Further, in the presence of low levels of S-adenosylmethionine, DNA MTase may be able to catalyze the deamination of DNA-cytosine to form uracil, leading to C to T transitions (Yang et al., 1995). Thus, the high rate of mutation at CpG dinucleotides may be due, in part, to DNA MTase-mediated deamination (Laird et al., 1995; Yang et al., 1995) and inhibition of DNA mismatch repair (Yang et al., 1995). Additionally, 5MeC may influence carcinogenesis via inhibition of DNA repair, leading to the fixation of a promutagenic lesion (references can be found in Counts and Goodman, 1994, 1995). However, the high percentage of mutations at CpG sites cannot be explained solely by the presence of 5MeC, as the methylation pattern of the p53 gene is tissue independent, suggesting that tissue-specific methylation does not contribute to the differential mutation patterns at CpG sites seen in tumors (Tornaletti and Pfeifer, 1995). This observation supports the contention that the overall effect of DNA methylation alterations may vary in different tissues and in the genesis of different tumor types.

The complex nature of the role of methylation in regulation of gene expression is illustrated by the *Igf2r* gene. For expression to occur, both hypomethylation of the 5′ flanking region and methylation of a specific CpG site in an intron are required. The latter appears to be an imprinting signal, and hypomethylation at this site may silence the gene even if the 5′ flanking region remains hypomethylated (Stöger et al., 1993).

Conclusions

This discussion of what at first glance might appear to be conflicting roles for DNA methylation in carcinogenesis actually enhances our understanding of the process. Further, the traditional view that the key mutations in cancers stem from carcinogen–DNA adducts is too narrow. The current literature provides a compelling basis for suggesting that mutations arising secondary to deamination of 5MeC, C, or both are an important source of critical point mutations. Mutation, altered gene expression, hypomethylation, and hypermethylation may all play roles in carcinogenesis (Figure 1); they are not mutually exclusive. We do not anticipate a simple one-to-one relationship between DNA methylation and cancer, mutation and cancer, or cell proliferation and cancer, nor do we anticipate all tissues to have identical mechanisms operative. In some situations hypomethylation may be most important, in oth-

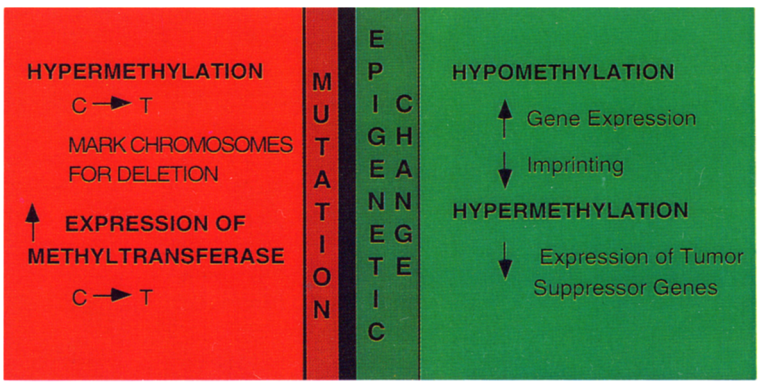

Figure 1. Multiple Roles for DNA Methylation in Carcinogenesis

See text for details.

ers hypermethylation, and in others mutation. The examination of DNA methylation status provides the potential to discover alterations in gene expression, cell proliferation, mutation, chromatin aberrations, and inactivation/deletion of tumor suppressor genes in one multifaceted approach that fits with the multistep process of carcinogenesis. In support of this notion is the depiction of human colon carcinogenesis, in which roles for hypomethylation of DNA, mutation, and tumor suppressor gene inactivation are considered to be relevant to the ultimate tumor formation (references can be found in Counts and Goodman, 1994, 1995). It is important that a minimalistic approach not be taken in defining the role of DNA methylation in carcinogenesis, as this runs counter to the established view of the transformation of a normal cell into a frank malignancy as being a multistep/multistage process. Indeed, one should assume that multiple mechanisms underlie carcinogenesis and must consider that a carcinogen may act by more than one mechanism.

Most importantly, investigations into the role of DNA methylation in carcinogenesis serve as a focal point for enhancing our understanding of the interplay between genetic and epigenetic factors underlying this disease process. There is a need to address the functional significance of specific changes in methylation (e.g., how the binding of *trans*-acting factors to specific genes is affected by methylation), the functional significance of DNA MTase-facilitated mutations in specific genes, and the functional significance of changes in methylation that occur in target tissues prior to the appearance of frank malignancies. The overall goal should be an understanding of changes in methylation and how they facilitate movement of cells through the different stages of carcinogenesis. This can be accomplished by keeping in perspective the fact that cancer is a disease of the whole animal, and thus there is a need to focus, though not exclusively, on in vivo studies. A variety of in vivo model systems are being employed productively in researching the role of methylation in carcinogenesis. These include studies involving methyl-deficient diets (references can be found in Counts and Goodman, 1994, 1995), DNA MTase mutants (Li et al., 1993; Laird et al., 1995), and animals that exhibit a genetic susceptibility toward tumor development (references can be found in Counts and Goodman, 1994, 1995; Laird et al., 1995). A key issue centers around discerning changes in methylation that are causative in the cancer process and distinguishing these from changes that may simply be correlative. It appears to us that progress in this area would be enhanced markedly if individual researchers could bring their expertise to bear on multiple aspects of DNA methylation and carcinogenesis using organs obtained from a common pool of experimental animals and in this way to examine hypomethylation, hypermethylation, and mutation in the same animals at different tissue sites.

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